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## Antibacterial Potential of Dewandaru Leaves (*Eugenia uniflora* L.) Against *Escherichia coli*: In Vitro Study

Riyan Setiyanto<sup>1\*</sup>, Annisa Diyan Meitasari<sup>2</sup>, Sinta Cantika Natasya<sup>3</sup>, Dewi Weni Sari<sup>1</sup>

<sup>1</sup>Lecturer, Diploma III of Pharmacy, Politeknik Indonusa Surakarta, Surakarta, Indonesia

<sup>2</sup>Lecturer, Diploma III of Pharmacy, Universitas Sebelas Maret, Surakarta, Indonesia

<sup>3</sup>Student, Diploma III of Pharmacy, Politeknik Indonusa Surakarta, Surakarta, Indonesia

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#### \*Corresponding author:

Riyan Setiyanto

#### E-mail address:

[riyansetiyanto@poltekindonusa.ac.id](mailto:riyansetiyanto@poltekindonusa.ac.id)

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### A B S T R A C T

**Introduction:** *Escherichia coli* is a pathogenic Gram-negative bacteria that commonly causes digestive tract infections, including diarrhea. Continuous use of antibiotics has led to an increase in bacterial resistance. Dewandaru leaves (*Eugenia uniflora* L.) have been known to have antibacterial activity against several pathogenic bacteria. This study aims to evaluate the antibacterial activity of dewandaru leaf extract against *E. coli* in vitro. **Methods:** Dewandaru leaves were extracted using the maceration method with 96% ethanol. The antibacterial activity test was carried out using the Kirby-Bauer disk diffusion method with gentamicin as a positive control and sterile NaCl as a negative control. The diameter of the inhibition zone was measured and analyzed statistically. **Results:** Dewandaru leaf extract shows antibacterial activity against *E. coli* with a significant zone of inhibition. An extract concentration of 80% showed moderate antibacterial activity, while concentrations of 40% and 60% did not show antibacterial activity. **Conclusion:** Dewandaru leaves have the potential to be a natural antibacterial agent against *E. coli*. Further studies are needed to isolate and identify the active compounds and evaluate the effectiveness and safety of dewandaru leaf extract in vivo.

### 1. Introduction

Gastrointestinal tract infections caused by the bacteria *Escherichia coli* (*E. coli*) have become a frightening specter throughout the world. It is not only limited to developing countries but also poses a serious threat to developed countries. The ability of this bacterium to cause diarrhea, especially in vulnerable groups such as children and individuals with weakened immune systems, has made it one of the main causes of global morbidity and mortality. The World Health Organization (WHO) has noted that diarrhea is the second cause of death in children under five worldwide, with *E. coli* as one of the main

causative agents. Infection of *E. coli* can cause severe dehydration, electrolyte disturbances, kidney damage, and even death if not treated quickly and appropriately. Additionally, some strains of *E. coli* pathogenic, such as *E. coli* producing Shiga toxin (STEC), can cause serious complications such as hemolytic uremic syndrome (HUS), which can be fatal. Widespread and often inappropriate use of antibiotics has fueled a global antibiotic resistance crisis. *E. coli*, like many other bacterial pathogens, has developed resistance to a wide range of antibiotics, including those considered the last line of defense. This resistance not only complicates the treatment of

infections of *E. coli*, but also increases the risk of complications, costs of care, and economic burden for individuals and the health system. This situation has encouraged researchers and health practitioners to look for effective and safe alternative therapies to treat infections of *E. coli*. One promising approach is exploring the potential of traditional medicinal plants as a source of natural antibacterial agents. Medicinal plants have been used for centuries by various cultures around the world to treat various ailments, including bacterial infections.<sup>1-3</sup>

Among the various medicinal plants available, dewandaru (*Eugenia uniflora* L.) has attracted the attention of researchers due to its rich phytochemical content and pharmacological potential. This plant originates from South America and has been introduced to various tropical regions, including Indonesia. Dewandaru leaves have been used traditionally to treat various ailments, including infections, inflammation, and digestive disorders. Several studies have revealed the antibacterial activity of dewandaru leaves against several pathogenic bacteria, such as *Streptococcus pneumoniae* and *Shigella dysentery*. Bioactive compounds thought to play a role in this antibacterial activity include flavonoids, tannins, and terpenoids. These compounds have various mechanisms of action, such as damaging bacterial cell membranes, inhibiting protein synthesis, and disrupting bacterial metabolic pathways. Although the antibacterial potential of dewandaru leaves has been demonstrated against several bacteria, its activity against *E. coli* is still not much explored.<sup>4-7</sup> Considering the urgency of the health problems caused by *E. coli* and increasing antibiotic resistance, further research into the antibacterial potential of dewandaru leaves against *E. coli* is indispensable. Therefore, this study aims to evaluate in depth the antibacterial activity of dewandaru leaf extract against *E. coli* in vitro.

## 2. Methods

The materials used in this research include fresh dewandaru leaves, 96% ethanol, bacterial culture *Escherichia coli* ATCC 25922, Mueller Hinton Agar (MHA) media, gentamicin (positive control), sterile

0.9% NaCl (negative control), sterile disc paper, and standard laboratory equipment. Fresh dewandaru leaves are washed, dried, and then crushed. Dewandaru leaf powder was extracted using the maceration method with 96% ethanol for 3 days. The filtrate resulting from maceration is concentrated using a rotary evaporator until a thick extract is obtained. The extract was then diluted with 96% ethanol in concentrations of 40%, 60%, and 80%.

The antibacterial activity test was carried out using the Kirby-Bauer disk diffusion method. Bacterial culture *E. coli* ATCC 25922 was grown in MHA media and incubated for 24 hours at 37°C. The bacterial suspension was made with a concentration of 0.5 McFarland. The bacterial suspension is then spread evenly on the surface of the MHA media using a sterile cotton swab. Sterile paper discs were dipped in dewandaru leaf extract with concentrations of 40%, 60%, and 80%. The dipped paper disc is then placed on the surface of the MHA media which has been inoculated with bacteria *E. coli*. Gentamicin was used as a positive control, while sterile 0.9% NaCl was used as a negative control. After incubation for 24 hours at 37°C, the diameter of the inhibition zone around the paper disc was measured using a caliper. Measurements were carried out three times for each extract concentration. The data obtained were analyzed statistically using the ANOVA test and Tukey's test.

## 3. Results and Discussion

Results of the antibacterial activity test of dewandaru leaf extract against *E. coli* are presented in Table 1. Dewandaru leaf extract with a concentration of 80% showed moderate antibacterial activity against *E. coli* with an average zone of inhibition of 10.33 mm. Concentrations of 40% and 60% did not show antibacterial activity against *E. coli*. Gentamicin as a positive control showed the largest zone of inhibition, namely 25.67 mm. ANOVA test showed significant differences between treatment groups ( $p < 0.05$ ). The Tukey test showed that there was a significant difference between the 80% concentration of dewandaru leaf extract and the negative control ( $p < 0.05$ ).

Table 1. Diameter of the inhibitory zone of dewandaru leaf extract against *E. coli*.

Extract concentration	Average inhibition zone (mm)	Standard deviation
40%	10.31	2.32
60%	10.32	1.67
80%	15.33	1.58
Gentamicin (positive control)	25.67	1.15
NaCl 0.9% (negative control)	0	0

The results of this study clearly show that dewandaru leaf extract (*Eugenia uniflora* L.) has antibacterial activity against *E. coli*, especially at a concentration of 80%. These findings strengthen existing scientific evidence regarding the potential of this plant as a natural antibacterial agent. The inhibition zone formed around the disc containing dewandaru leaf extract at a concentration of 80% indicates the presence of bioactive compounds capable of inhibiting growth and proliferation. *E. coli*. Antibacterial activity of dewandaru leaves against *E. coli* This is in line with the results of previous studies showing similar effects on other pathogenic bacteria, such as *Streptococcus pneumoniae* and *Shigella dysenteriae*. The consistency of these findings strengthens the notion that dewandaru leaves have a broad spectrum of antibacterial activity, making them potential candidates for the development of natural antibacterial agents that can treat various types of bacterial infections.<sup>8-12</sup>

The antibacterial activity of dewandaru leaves is thought to be closely related to the abundant content of bioactive compounds, especially flavonoids, tannins, and terpenoids. These compounds are known to have diverse mechanisms of action against bacteria, which synergistically contribute to the observed antibacterial effects. The antibacterial activity of dewandaru leaves is thought to be closely related to the abundant content of bioactive compounds, especially flavonoids, tannins, and terpenoids. These compounds are known to have diverse mechanisms of action against bacteria, which synergistically contribute to the observed antibacterial effects. This group of polyphenolic compounds is one of the key components in the antibacterial activity of dewandaru leaves. Flavonoids have the ability to disrupt various vital processes in bacterial cells, inhibiting their growth and proliferation. Flavonoids can interact with

key enzymes involved in bacterial DNA replication and transcription. For example, some flavonoids can inhibit the activity of DNA gyrase and topoisomerase IV, which play an important role in unwinding and rewinding DNA during replication. By inhibiting these enzymes, flavonoids can prevent bacterial DNA replication, inhibit their growth, and ultimately cause cell death. Flavonoids can also interact with bacterial cell membrane lipids, disrupting membrane integrity and permeability. This can cause leakage of cell contents, ionic imbalance, and disruption of vital cellular functions, ultimately leading to the death of the bacteria. Some flavonoids can even cause the formation of pores in cell membranes, which accelerates the process of cell leakage and death. Apart from DNA gyrase and topoisomerase IV, flavonoids can also inhibit the activity of other enzymes that are important for bacterial survival, such as enzymes involved in energy metabolism and protein synthesis. By inhibiting these enzymes, flavonoids can disrupt the cellular function of bacteria to a large extent and inhibit their growth. Flavonoids can also work synergistically with other bioactive compounds in dewandaru leaves, such as tannins and terpenoids, to increase the overall antibacterial effect. For example, flavonoids can increase the permeability of bacterial cell membranes, making it easier for other compounds to enter cells and disrupt bacterial cellular processes.<sup>13-18</sup>

Tannins, as a group of polyphenolic compounds, have a crucial role in the defense mechanism of dewandaru leaves against bacterial infections. Its unique ability to bind to proteins makes it a powerful weapon in disrupting various vital processes in bacterial cells. One of the main targets of tannins is bacterial cell membranes. Tannins can interact with membrane proteins, especially transmembrane proteins which play a role in maintaining membrane

integrity and function. This interaction can disrupt membrane structure, cause changes in permeability, and increase the risk of leakage of cell contents. As a result, bacteria lose the ability to maintain their internal homeostasis, experience ionic imbalance, and ultimately die. Apart from that, tannins can also interfere with the process of transporting nutrients across cell membranes. By inhibiting transport proteins, tannins can prevent bacteria from getting the essential nutrients needed for growth and survival. Tannins have a high affinity for proteins, including enzymes that play an important role in bacterial metabolism. Tannins can bind to and inhibit the activity of these enzymes, disrupting metabolic pathways important for energy production, cellular component synthesis, and DNA replication. For example, tannins can inhibit enzymes involved in glycolysis, the citric acid cycle, and oxidative phosphorylation, which are the main pathways for energy production in bacterial cells. By inhibiting energy production, tannins can weaken bacteria and inhibit their growth. Apart from that, tannins can also inhibit enzymes involved in protein synthesis, such as ribosomes and enzymes that play a role in translation. Inhibition of protein synthesis can interfere with the formation of new cellular components, inhibit growth, and cause bacterial death. Tannins not only work alone but can also work synergistically with other bioactive compounds in dewandaru leaves, such as flavonoids and terpenoids. For example, tannins can increase the permeability of bacterial cell membranes, making it easier for flavonoids and terpenoids to enter cells and interfere with other cellular processes. The combined effects of these various bioactive compounds create a multi-target attack against bacteria, increasing the overall antibacterial effectiveness of dewandaru leaves. Tannins, with their unique ability to interact with proteins, play an important role in the defense mechanism of dewandaru leaves against bacterial infections. By disrupting cell membrane integrity, inhibiting enzyme activity, and working synergistically with other bioactive compounds, tannins contribute significantly to the antibacterial activity of dewandaru leaves against *E. coli*.<sup>19-24</sup>

Terpenoids, as a very diverse group of organic compounds, also contribute strength to the antibacterial arsenal of dewandaru leaves. The structural and functional diversity of terpenoids allows them to attack bacteria through various mechanisms, complementing the action of flavonoids and tannins. Several types of terpenoids, especially monoterpenes and sesquiterpenes, have the ability to damage bacterial cell membranes. This mechanism is similar to the way flavonoids work, namely by disrupting the integrity of the lipid bilayer membrane. Terpenoids can interact with membrane lipids, changing their fluidity and permeability. This can cause leakage of cell contents, ionic imbalance, and disruption of vital cellular functions, ultimately leading to bacterial death. Terpenoids can also interfere with the process of bacterial protein synthesis, which is a crucial process for the growth and survival of bacteria. Some terpenoids can interact with ribosomes, which are the molecular machines responsible for translating genetic code into proteins. By inhibiting ribosome function, terpenoids can prevent the formation of important proteins that bacteria need to survive. Apart from that, some terpenoids can also inhibit enzymes involved in the translation process, namely the process of forming polypeptide chains from amino acids. By inhibiting these enzymes, terpenoids can disrupt overall protein synthesis, cause disruption of cellular function, and inhibit bacterial growth.<sup>25-27</sup>

Bacteria communicate and coordinate through complex signaling pathways, involving signal molecules and receptors. This signaling pathway is important for a variety of bacterial physiological processes, including growth, differentiation, biofilm formation, and virulence. Some terpenoids are known to interfere with bacterial signaling pathways by interacting with receptors or signal molecules. By disrupting communication between bacterial cells, terpenoids can inhibit growth, reduce virulence, and prevent the formation of biofilms, which are bacterial communities that are resistant to antibiotics. The flavonoids, tannins, and terpenoids in dewandaru leaves work together synergistically to create a strong and effective antibacterial effect against *E. coli*.

Flavonoids and terpenoids can disrupt cell membrane integrity and inhibit protein synthesis, while tannins can potentiate these effects by disrupting membrane function and inhibiting enzymes. The combination of these various mechanisms of action makes it difficult for bacteria to develop resistance so dewandaru leaves are a promising candidate for the development of effective and safe natural antibacterial agents.<sup>26-29</sup>

Although dewandaru leaf extract shows significant antibacterial activity against *E. coli*, the effect was lower than that of gentamicin, which is the standard antibiotic used as a positive control in this study. This can be explained by several factors. First, gentamicin is an aminoglycoside antibiotic which has a very specific and strong mechanism of action against bacteria. Gentamicin binds irreversibly to the 30S subunit of bacterial ribosomes, inhibits protein synthesis, and causes bacterial death. This mechanism of action is highly effective against various types of bacteria, including *E. coli*. Second, dewandaru leaf extract is a complex mixture of various bioactive compounds, each of which has a different mechanism of action and potency. Although some compounds in dewandaru leaf extract can have strong antibacterial activity, their effect can be diluted by other compounds that are less active or even inactive. Third, the concentration of dewandaru leaf extract used in this study may not have been optimal. Further research is needed to determine the optimal concentration that can produce a stronger antibacterial effect.<sup>25-28</sup>

Even though its antibacterial activity is lower than gentamicin, the potential of dewandaru leaves as a natural antibacterial agent remains significant. This is especially relevant considering the increasing resistance of bacteria to conventional antibiotics. dewandaru leaves can be a promising therapeutic alternative, especially in cases of infection *E. coli* mild treatment or as a complementary therapy along with conventional antibiotics. As an alternative therapy, dewandaru leaves can be used in the form of herbal preparations, such as infusions or decoctions, to treat infections of *E. coli* in the digestive tract. The use of dewandaru leaves as a complementary therapy can help increase the effectiveness of conventional

antibiotics and reduce the risk of bacterial resistance.<sup>28,29</sup>

The results of this research open up opportunities for further research to explore the potential of dewandaru leaves as a natural antibacterial agent. Further research is needed to isolate and identify the specific compounds in dewandaru leaves that are responsible for their antibacterial activity against *E. coli*. This can be done using various chromatographic and spectroscopic techniques. In-depth research regarding the mechanism of action of the active compounds of dewandaru leaves on *E. coli* is necessary. This can include the study of the interaction of active compounds with bacterial molecular targets, such as cell membranes, ribosomes, and important enzymes. The effectiveness and safety of dewandaru leaf extract in treating infections of *E. coli* needs to be evaluated in experimental animals and humans. In vivo, studies can provide important information regarding the effective dose, pharmacokinetics, and toxicity of dewandaru leaf extract. If proven effective and safe, dewandaru leaf extract can be developed into a pharmaceutical preparation that is more standardized and easy to use, such as capsules, tablets, or ointments. With further comprehensive research, dewandaru leaves have the potential to become a valuable source of natural antibacterial agents to overcome the problem of antibiotic resistance and improve public health.<sup>30</sup>

#### **4. Conclusion**

Dewandaru leaf extract shows antibacterial activity against *E. coli*, especially at a concentration of 80%. Dewandaru leaves have the potential as a natural antibacterial agent that can be used as an alternative or complementary therapy to treat infections of *E. coli*. Further research is needed to explore this potential in more depth.

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